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# X-ray absorption spectroscopy studies of ubiquinol oxidase membrane protein

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We report the X-ray absorption spectroscopy studies of the Cu and Fe edges of the cytochrome bo3 quinol oxidase from Escherichia coli at the room temperature. Heme-copper oxidases are integral membrane proteins in which proton pumping takes place. Most proposed proton-pumping mechanisms involve CuB and its histidine ligands. The existence and identity of such reorganization of the CuB geometry caused by protonation/deprotonation and/or breakage of one of the Cu-N(His) bonds is a difficult matter to either prove or disprove since CuB is spectrally silent.
The experimental setup which combined the optical microspectrometer and X-ray absorption measurements in fluorescence mode has been tested. The optical microspectrometer has been used to control the photoreduction of the protein sample. Our experimental results shown that at the iron K-edge the photoreduction of the sample happened very fast (dozens seconds) otherwise at the copper K-edge the photoreduction occurred after several minutes that allowed us to measure the XANES part without using a cryojet.
Final results indicate that CuB varied its associated ligands for oxidised Cu(II) and reduced Cu(I) states of the protein. However room temperature copper K-edge X-ray absorption spectra remains unchanged in the pH range 6.5-9.5 for both oxidised and reduced forms of copper correspondently, indicating that no structural changes takes place at CuB depending on pH.

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